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LITERATURE CITED

1. J. Miller, Experiments in Molecular Genetics, Cold Spring Harbor (1972).
2. A. G. Skavronskaya et al., Dokl. Akad. Nauk SSSR, 236, 460 (1977).
3. L. M. Fonshtein et al., in: A Test System for Evaluation of Mutagenic Activity of Environmental Pollutants on Salmonella. Technical Instruction [in Russian], Moscow (1977), p. 19.
4. B. N. Ames, W. E. Durston, E. Yamasaki, et al., Proc. Natl. Acad. Sci. USA, 70, 2281 (1973).
5. B. A. Bridges, in: Environmental Mutagens, H. Böhme and J. Schoneich, eds., Berlin (1977), p. 9.
6. P. Caillet-Fauquet, M. Defais, and M. Radman, J. Mol. Biol., 117, 95 (1977).
7. F. Haas and C. Doudney, Proc. Natl. Acad. Sci. USA, 43, 871 (1957).
8. R. W. Hart and R. B. Setlow, in: Molecular Mechanisms for Repair of DNA, P. C. Hanawalt and R. B. Setlow, eds., Part 6, New York (1975), p. 719.
9. J. McCann, N. E. Spingarn, Y. Kobori, et al., Proc. Natl. Acad. Sci. USA, 72, 979 (1975).

EFFECT OF THIOPHOSPHAMIDE ON THE FREQUENCY OF CHROMOSOMAL ABERRATIONS IN ATAXIA-TELANGIECTASIA HETEROZYGOTES

Yu. E. Ivanova, Academician N. P. Bochkov,* and K. N. Yakovenko

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Ataxia-telangiectasia (AT), or the Louis–Bar syndrome, is an autosomal-recessive hereditary disease with severe progressive cerebellar ataxia, disturbances of immunity, and telangiectasias in the cornea and skin, and it belongs to the group of syndromes with chromosomal instability. The patients show an increased spontaneous level of chromosomal aberrations [1, 5] and predisposition to malignant neoplasms [8]. On the question of heterozygous carriers of the AT gene a report has been published describing some increase in the spontaneous level of chromosomal aberrations in lymphocyte cultures and, in particular, in fibroblast cultures [7]. Induced mutagenesis has not been studied in these cultures. Considering the fact that persons heterozygous for AT are fairly widespread in the population (about 1%) [6], it was decided to study the principles governing induced mutation in their cells.

The frequency and spectrum of chromosomal aberrations were compared in cultures of lymphocytes from two groups of donors (heterozygotes for AT and subjects of the control group), exposed to different doses of thiophosphamide.

EXPERIMENTAL METHOD

Cultures of peripheral blood lymphocytes from six heterozygous carriers of the AT gene (parents of patients with ataxia-telangiectasia) and from six healthy donors were used. The experiments were carried out within a period of 6 months as the patients were admitted, and one essential condition was observed: simultaneously with the lymphocyte culture of the subject heterozygous for AT, a lymphocyte culture of a control donor was used in the experiment. This was because the true concentration of commercial thiophosphamide

*Academy of Medical Sciences of the USSR.

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TABLE 1. Action of Different Concentrations of Thiophosphamide on Lymphocyte Cultures of Subjects Heterozygous for AT and Control Donors

Concentration of thiophosphamide, μg/ml	Individual	Metaphases with chromosomal aberrations, %	
		in heterozygotes for AT	in control donors
10	I	13	7
	II	31	16
	III	21	15
	IV	30	22
	V	11	18
	VI	19	22
15	I	25	19
	II	49	36
20	I	37	23
	II	61	42
	III	44	37
	IV	48	39
	V	56	44
	VI	39	38
25	I	34	41
	II	64	55
30	I	41	58
	II	79	70
	III	59	58
	IV	69	63
	V	71	63
	VI	53	58

TABLE 2. Results of Regression Analysis of Concentration Dependences (M ± m)

Indices	Heterozygotes for AT	Control donors
Fraction of scatter, demonstrated by equation	0,4495	0,8361
Coefficients of regression equation:		
$a \pm s$	0,3015±0,4418	0,0319±0,2443
$b \pm s$	0,0231±0,0208	0,0319±0,0115
F of inadequacy	0,0980	0,1971
F of regression	6,5327	40,8013

differs in different batches put on the market. When the experiments were carried out in this way the concentration of the mutagen was always the same for the heterozygous and control donors.

Blood was treated with thiophosphamide in concentrations of between 10 and 30 μg/ml for 1 h before the beginning of culture. The mutagen was thoroughly washed off. Cultures of lymphocytes from the I and II pairs of individuals (the heterozygous carrier of the AT gene and the control donor) were treated with thiophosphamide in five concentrations (10, 15, 20, 25, and 30 μg/ml), those of the III and IV pairs were treated with thiophosphamide in three concentrations (10, 20, and 30 μg/ml). The conditions of culture, preparation and staining of the films, and the principles of cytogenetic analysis were those usually adopted. The cells were fixed at the 58th hour of culture. Colchicine was added 2 h before fixation. The specimens were numbered; at each point 100 cells were analyzed, making 5500 cells altogether. The frequency of cells with aberrations and the ratio between the different types of chromosomal structural changes were estimated.

The results were subjected to statistical analysis by dispersion [3] and regression [2] methods.

EXPERIMENTAL RESULTS

The spontaneous frequency of cells with chromosomal aberrations in the control donors was 2.0 ± 2.92, and in the subjects heterozygous for AT it was 2.83 ± 2.23. Statistically significant differences between the groups were not found ($t = 0.54$; $n = 9$).

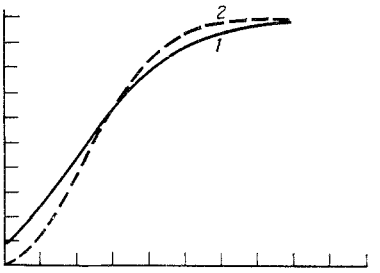

Fig. 1. Action of thiosphosphamide on culture of lymphocytes from heterozygotes for AT. Abscissa, thiosphosphamide concentration (in μg/ml); ordinate, percentage of aberrant metaphases. 1) Theoretical curve showing dependence of fraction of aberrant metaphases on thiophosphamide concentration in cells of heterozygotes for AT; 2) the same in cells from control donors.

Data showing the frequency of cells with aberrations after exposure to thiophosphamide are given in Table 1.

Dispersion analysis, undertaken for the experiments with concentrations of 10, 20, and 30 μg/ml, showed that the frequency of cells with chromosomal aberrations in the lymphocyte cultures from the two groups of donors differed ($F = 7.47$; $n = 1$; $0.01 < P < 0.05$). Lymphocytes of heterozygotes for AT were more sensitive to the action of thiophosphamide than lymphocytes of the control donors.

Comparison of the concentration dependences in the two groups of donors was carried out for I and II pairs of individuals and for treatment with thiophosphamide in five concentrations.

To analyze the concentration dependence existing data showing that the shape of the curves for the action of thiophosphamide on cultures of lymphocytes from the healthy donor described by the equation:

$$\rho = 1 - e^{(-a+bC)^2},$$

where ρ is the proportion of injured metaphases, C the concentration of thiophosphamide, and a and b are parameters of the equation, were used as the starting point [4]. Statistical analysis of the data by the method of regression analysis showed that this same equation satisfactorily describes the shape of the concentration curves for the action of thiophosphamide on lymphocyte cultures from heterozygotes for AT (Table 2, Fig. 1).

Extrapolation to the zero point ($C = 0$ μg/ml) by means of this equation for the group of heterozygotes led to a higher than expected level for spontaneous chromosomal aberrations ($\rho_0 = 0.0869 \pm 0.1773$), which was not observed in the control group ($\rho_0 = 0.0010 \pm 0.0579$). In this case ρ_0 is the theoretically calculated spontaneous level of chromosomal aberrations. Since no differences were observed in the frequency of spontaneous chromosomal aberrations between the two groups of donors, it can be tentatively suggested that in heterozygotes for AT the sensitivity of the lymphocyte cultures to the action of low concentrations of thiophosphamide is increased, and this leads to a higher than the theoretically expected spontaneous level of chromosomal aberrations.

Dispersion analysis of the data for the ratio between the types of chromosomal aberrations (exchanges, single fragments, paired fragments) in the heterozygotes for AT and the control donors showed the absence of statistically significant differences in the spectrum of chromosomal aberrations between the two groups of donors ($P > 0.05$).

Differences between heterozygotes for AT and control donors in the reaction to the mutagens were thus manifested only in the frequency of cells with chromosomal aberrations. It is not yet possible to judge the nature of the differences discovered. The results obtained can, on the one hand, form an addition to the characteristics of ataxia-telangiectasia as a hereditary disease and, on the other hand, they may prove useful for discussion of the causes of the increased frequency of malignant neoplasms in heterozygotes for AT [9].

LITERATURE CITED

1. N. P. Bochkov, Yu. M. Lopukhin, N. P. Kuleshov, et al., Genetika, No. 6, 130 (1974).
2. N. R. Draper and H. Smith, Applied Regression Analysis, Wiley, New York (1966).

3. C. Hicks, Basic Principles of the Planning of Experiments [Russian translation], Moscow (1967).
4. A. N. Chebotarev and K. N. Yakovenko, Genetika, No. 8, 150 (1974).
5. N. P. Bochkov, Yu. M. Lopukhin (Y. M. Lopukhin), N. P. Kuleshov, et al., Humangenetik, 17, 91 (1973).
6. E. Boder, in: Handbook of Clinical Neurology, edited by P. J. Vinken and G. W. Bruyn, Vol. 14, Amsterdam and New York (1975), p. 267.
7. M. M. Cohen, M. Shaham, J. Dagan, et al., Cytogenetics, 15, 338 (1975).
8. F. Hecht and B. K. McCaw, in: Genetics of Human Cancer, J. J. Mulvihill et al., eds., New York (1977), p. 105.
9. M. Swift, L. Sholman, M. Perry, et al., Cancer Res., 36, 209 (1976).